

RESEARCH ARTICLE

URINE CYTOLOGY SCREENING FOR BLADDER CANCER AMONG TOBACCO CIGARETTE SMOKERS IN CALABAR METROPOLIS

I J. Inyang¹, I E. Bassey², M Udonkang¹, C. Ugori³, C. Udoka³

¹ Lecturer 2, Medical Laboratory Science Department, University of Calabar, Calabar, Nigeria

² S. Lecturer, Medical Laboratory Science Department, University of Calabar, Calabar, Nigeria.

³ Principal medical laboratory scientists, pathology department, University of Calabar teaching hospital, calabar, Nigeria.

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ABSTRACT: **Introduction:** This is a descriptive study carried out in Calabar municipal and Calabar South local governments, Cross River State, during the period of April (2015) to December (2015). **Aim:** The main purpose of carrying out this research work is to evaluate the cytomorphological features of urine smears (using papanicoulaou stain) among cigarette smokers in Calabar rcigarette smokers while 53 subjects were non- cigarette smokers. The numbers of years of cigarette smoking were different and the numbers of cigarette sticks smoked per day were also variable among the test group. Individuals with urinary tract infection were not included in this research work and individuals with less than three (3) years of cigarette smoking were also not included in this research work. From each urine sample collected, smears were obtained from the sediments after centrifuging and were immediately fixed with a cytology-spray fixative for at least 30 minutes, before staining smears with Papanicolaou stain. **Results and Discussion:** The stained smears were examined under a light microscope and revealed a high cellular turnover among 70% of the test group when compared with the control groups which are nonsmokers, showing few normal urothelia cells. Enlargement in nuclear cytoplasm ratio, irregular nuclear borders, necrosis, cluster of cells showing dysplastic changes, moderate haemorrhage, heavy infiltrates of inflammatory cells, hyperchromatism, pleomorphysms and neoplastic transformation were among the features observed in smears of the test group. **Conclusion:** On the basis of this research work, cigarette smoking has been seen to be one of the leading causes of bladder cancer in line with findings of Humidah A., Abdelhabib, Laura P. and Witold C. (2014).

KEYWORDS: Cytomorphological, cigarettes, papanicolaou, pleomorphysm, Urine cytology .

INTRODUCTION:

Bladder cancer accounts for majority of the urothelial tumours (NNSCR (2016), Ploeg M., Aben K. & Kiemeney L. (2009), Torre LA., Bray

F., Siegel RL., et al. (2015). Others include kidney and ureter cancers. The major risk factor associated with the development of bladder cancer

Corresponding Author:
IMEOBONG J INYANG,
Medical Laboratory Science Department, University of Calabar, Calabar, Nigeria.
Email- onyx294@gmail.com



is tobacco cigarette smoking (Besaratinia et al., 2013; Boffeta, 2008). Other confounding factors include age, gender, race and ethnicity, prior chemotherapy, genetic or family history of urothelial tumours, chronic bladder irritation and infections (American Cancer Society, 2018). Tobacco cigarette smoking is a common habit in rural and urban areas of Nigeria (Okafor & Okoroiwu, 2017). In Calabar, there is increase in the number of smokers especially among adolescents (Odey et al., 2012). In addition, Inyang et al. 2013 established a weak association between tobacco smoking and the development of urothelial neoplasia among furniture workers.

Cigarette smoking is known to cause about 50% of bladder cancer (Freedman et al., 2011) and smokers have a 2-fold to 6-fold risk of having bladder cancer than non-smokers (Torre et al., 2015). About 90% of bladder cancers caused by smoking are transitional cell carcinomas also called urothelial carcinomas (Torre et al., 2015; Takure et al., 2015). These bladder cancers are also likely to be of high-grade type at first diagnosis (Jiang et al., 2012). Baris et al. 2009 and Besaratinia et al. 2013 stated that this risk of bladder cancers from cigarette smoking is directly related to the intensity and duration of smoking.

Thus, this study was carried out to screen and assess the risk of bladder cancers in urine cytology smears of tobacco cigarette smokers in Calabar metropolis.

MATERIALS AND METHOD:

Study area

The study was carried out in Calabar metropolis the capital of Cross River State, located in South-South of Nigeria with coordinates $4^{\circ}57'0''N$ $8^{\circ}19'30''E$ by $4.95000^{\circ}N$ $8.32500^{\circ}E$. Two Local Government Areas, Calabar South and Calabar Municipal make up the metropolis. Calabar boost

of a population of 371million persons and an area of 406 kilometres (Eze & Effiong, 2010).

Study subjects

A total of 171 urine specimens were obtained in universal containers from 118 subjects (male smokers) and 53 controls (male non-smokers). The population was stratified and simple random sampling technique was used to select the subjects based on duration of smoking. Structured questionnaires were used to obtain demographic data and smoking related attitudes.

Inclusion Criteria

Subjects included for this study were people who have been smoking cigarettes for at least 3 years who also had unexplained blood in urine as well as burning during urination . Control subjects were males who had never smoked cigarette or use other tobacco products and with no medical history of any form of kidney disease.

Exclusion Criteria

Subjects who were not smoking cigarettes for a period of up to 3 years at the time of sample collection were excluded. Subjects with history of kidney diseases were also excluded from the study.

Ethical approval

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with Helsinki Declaration and the protocol was approved by the Ethics Committee of the Cross River State Ministry of Health (CRS/MH/HREC/015/Vol. vii)

Sample size calculation

Employing the sample size formula $N = Z^2 \times P \times q/d$ for single proportion, a 95% confidence level of prevalence of 18.1% (Odey, FA, Okokon, IB,

Ogbeche, JO et al. (2012) and a 5% allowable margin of error, a sample size of 171 was obtained.

Sample collection and processing

Mid stream random urine samples were collected from the subjects into 20ml sterile universal bottles. For each urine sample, 10ml was transferred into a sterile clean test tube, and was spun for 10minutes at 1500 rpm in a centrifuge. After centrifuging, the urine supernatant was discarded and the sediment was used to make a smear on a clean grease-free glass slide. The smear was immediately fixed with 95% ethanol for 30 minutes before staining with Papanicolaou method Ochei J. and Kolhartar A. (2005). All slides were viewed and photomicrographs captured with a LEICA digital microscope (DM-500) for cytomorphological changes. The urine cytology was reviewed and categorized in line with the Paris guidelines by Barkan G, Wojcik M., Savicfrume S. and Nayar R. (2016). The intensity of smoking was categorized based on method of Freedman et al. 2011.

Statistical analysis

Statistical package for the social sciences (SPSS) version 20 IBM Corporation, Armonk, New York was used to analyze the results. Chi-square was used to analyze prevalence. All results were statistically significant at probability level less than 0.05.

RESULTS:

Table 1 shows the age range of the test and control subjects. The highest number of subjects was found in the age range 31-40 years in both the smokers 32(27.1%) and non-smokers 27(50.9%). Subject aged 71-80 years had the least number of participants 1(2.5%) among the smokers. Fig. 1 shows the intensity of smoking per day. The highest number of smokers 55(46%) took 11-20

sticks per day and least number of smokers 5(4%) took the highest number of 31-40 sticks each day. Those who took the least number of 1-10 sticks were 48(41%). Table 2 shows the prevalence of bladder cancer among the subjects. Among the smokers, 7(5.9%) were non-diagnostic and 80(67.8%) were negative for high grade urothelial carcinoma (NHGUC), 22(18.6%) were atypical urothelial carcinoma (AUC), 7(5.9%) were suspected for high grade urothelial carcinoma (SHGUC) and 3(2.5%) were diagnosed of high grade urothelial carcinoma (HGUC). Among the non-smokers 18(33.9%) were non-diagnostic, 33(62.3%) were NHGUC, 2(3.8%) were AUC and none 0(0%) was suspected or diagnosed for high grade urothelial carcinoma. This difference was statistically significant ($\chi^2 = 27.175$, df (4), $p=0.001$). Plate 1 shows a photomicrograph positive for high grade urothelial carcinoma (HGUC) and Plate 2 shows a photomicrograph negative for high grade urothelial carcinoma (NHGUC).

Table 1: Age range of test group and control group

Age range (years)	Smokers n=118	Non-smokers n=53
	No. examined (%)	No. examined (%)
<20	7 (5.9)	10(18.9)
21-30	20(16.9)	12(22.6)
31-40	32(27.1)	27(50.9)
41-50	25(21.2)	3(5.7)
51-60	18(15.3)	1(1.9)
61-70	13(11.0)	0(0)
71-80	3(2.5)	0(0)
TOTAL	118(100)	53(100)

$\chi^2 = 31.258$ df = 6, $p = 0.001$

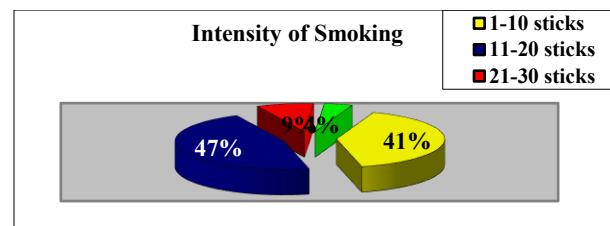


FIGURE.1: Intensity of smoking

Table 2: Prevalence of bladder cancer among smokers

	Smokers	Non smokers
Diagnostic criteria	No.(%)	No.(%)
Non diagnostic	7(5.9)	18(33.9)
NHGUC	80(67.8)	33(62.3)
AUC	22(18.6)	2(3.8)
SHGUC	7(5.9)	0(0)
HGUC	3(2.5)	0(0)
TOTAL	118(100)	53(100)

$\chi^2 = 27.175$, df (4), p= 0.001

Table 3 shows number of cigarette sticks smoked per day

RANGE OF STICKS	Frequency	Percentage (%)
<5	10	8.5
6-10	38	32.2
11-15	32	27.1
16-20	23	19.5
21-30	10	8.5
31and above	5	4.2
TOTAL	118	100

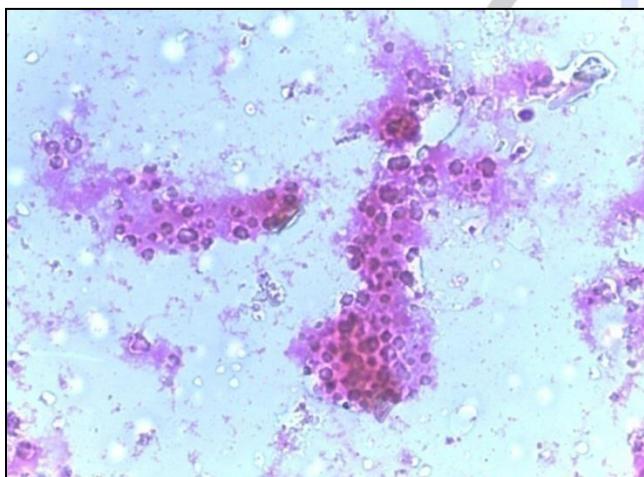


FIGURE 1: Urine smear of cigarette smoker positive for HGUC

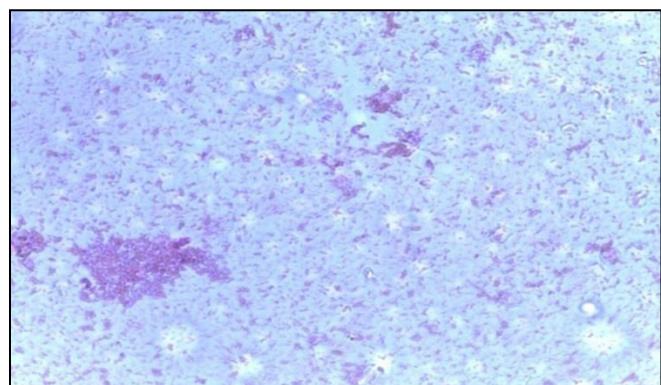


FIGURE 2: Urine smear of cigarette smoker with NHGUC X400. PAPANICOLOAU STAINING

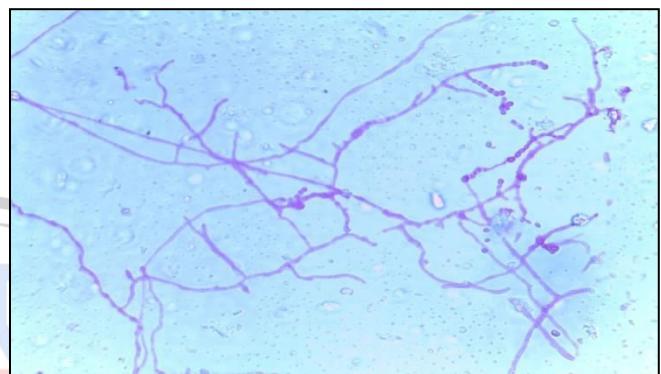


FIGURE 3: Test urine smear from cigarettes smokers showing fungal HYphae (MOLD SPORES)

DISCUSSION AND CONCLUSION:

In this study, urine smears were used to assess the effect of duration and intensity of smoking on bladder epithelial cytomorphology and risk of development of urothelial carcinoma among smokers of different age groups.(Erik Pasin et.al.,2008)

It was observed that a greater number of smokers were young adults below the age of 40 years. This trend shows that most of the subjects-initiated smoking at younger ages and became habitual smokers earlier. This is in line with reports from other works by Takure et.al. 2015

. The major reason behind this social trend is believed to be as a result of peer group influence. In Nigeria, there is weak regulation of the sales and promotion of cigarettes as it is sold in the open to minors. Also cigarette smokers puff up in open places and gatherings with no restriction. These social behaviors influence young people to initiate smoking at younger ages. This is worrisome and calls for effective implementation of the National Tobacco Act.

Also a greater number of the smokers 27.1% took at least 11-15 sticks/day consistent with report from Ajeleye et al., 2016. In this study duration of smoking (> 3years) and intensity of smoking (number of sticks/day) were found to be a predisposing factor to bladder cancer similar to report by Baris et al., 2009 and Besaratinia et al., 2013.

The cytomorphological features observed among the urine smears of cigarette smokers include increased in nuclear-cytoplasmic ratio, irregular nuclear border, nuclear hyperchromasia, pleomorphism, and dysplastic changes similar to report by Ajeleye et al., 2016. High grade urothelial carcinoma was diagnosed among the smokers but was negative among the non-smokers. This association of smoking with bladder cancer can be compared with reports by Pietzak et al., 2013; Cumberbatch et al., 2016 and Jiang et al., 2012.

The ability of cigarette smoke to cause bladder cancer is as a result of deposition of carcinogens on the bladder epithelium (Cumberbatch et al., 2016; Nooshinfar et al., 2017). Nicotine is one of the prominent compounds found in cigarette. Polycyclic aromatic hydrocarbons, aromatic amines, and N-nitro compounds are found in cigarette smoke. Accumulations of these compounds in the bladder epithelium coupled with slow or absent xenobiotic enzymes such as N-acetyltransferases and glutathione S-transferases

necessary for detoxification of these carcinogens leads to epithelium changes (Cumberbatch et al., 2016; Nooshinfar et al., 2017). These compounds cause adduct formation on DNA, DNA breaks, and modification of nucleotide bases, leading to DNA mutations and cancer (Besaratinia et al., 2013; Cumberbatch et al., 2016).

This study reveals that tobacco cigarette smoke causes cytomorphological changes in the bladder epithelial cells of subjects who have been smoking for at least 3 years and is associated with increased risk of development of bladder cancers including high grade urothelial carcinoma. Janet Raloff (2010) reported that tobacco in cigarette may be a source of infection as it hosts hundreds of different germs responsible for many human diseases although this has been severely criticized based on the argument that as tobacco burn, it kills any germs, but the tobacco farthest from the burning tip may be a balmy temperature for the germs. In this study, we observe a preponderance of fungal hyphae in the urine specimen of smokers that was absent in the urine specimens of non smokers (figure 3). Mold is a fungus which reproduces by producing spores. Mold spores are like seeds that thrive in poor ventilation in a temperature of between 40 and 110 degrees Fahrenheit (the smoking temperature). Mold travel by mold spores by hitch hiking on a host especially humans. Mold spores contain mycotoxins, mycotoxins are inhaled or absorbed into the skin when a high amount of airborne toxic mold are inhaled into the lungs, Its enter the blood stream where the mycotoxins become secondary metabolites causing disease such as urothelial carcinoma. There is need to fully implement the National Tobacco Law in Nigeria and continue to create awareness about the risk associated with smoking and benefits of quitting to maintain good health.

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Authors' contribution

IJI and IBE conceive, plan and carried out the work, authors CU and CU carried out laboratory procedures. MIU and ASO carried out statistical analysis. All authors read and edited the final article before submission.

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